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Polysome profiling of Cell Cycle

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Abstract

mRNA Translation is a highly regulated process and even more so during cell cycle transition where and the activation or degradation of proteins mediate progression through the various phases of the cell cycle. In numerous efforts have been made to study this regulation using genome-wide studies which include proteomic and ribo-seq approaches. These studies have shown that translation is not only globally regulated but is also gene specific for each phase of the cell cycle. However, the major drawback of these studies is the use of synchronisation to obtain the certain phase. It is unknown how the synchronisation might affect the gene regulation itself, despite certain studies showing changes in posttranslational modifications due to synchronisation. The research data also lacks any information about the influence of mRNA features like UTR length, structure, and composition on it is translatability.

Here, we coupled the well-established, high-sensitive polysome profiling method (Scarce sample polysome profiling; SSP-Profiling) with flow cytometry to obtain unperturbed cells from different phases of cell cycle and evaluate of their transcriptome and translatome. To achieve this, we used non-leukemic lymphoblastoid cell line, NCNC, with diploid karyotype. The cells were mildly fixed to be able to sort them before performing SSP- profiling. We demonstrate the applicability of this method, by sorting different number of cells, 20K, 50K and 100K and thus obtaining polysome profiles for each of them. This is also supported by Puromycin treated controls, which clearly show the dissociation of polysome indicating overall reliability of fixation, sorting and SSP-Profiling protocol and capability to monitor actual status of translation. Furthermore, each sample (G1, S and G2M) with 50K cells were fractionated to 6 polysome profile fractions corresponding to: loading peak, 40-60 S, 60-80 S regions of the profile and to light, medium and heavy polysomes and processed for RNA sequencing along with total RNA to obtain translation data of each individual mRNA. We identified numerous mRNAs encoding cell cycle regulated genes along with genes regulated at the translation level unique to lymphoblastoid cells. Presently, we are working with the functional characterisation of the same. The establishment of this method broadens the scope of translation study in biologically limited samples with the possibility of coupled flow assisted sorting.

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